

Article title

Effects of plastics and tire leachate on marine microalga *Dunaliella primolecta* and copepod *Tigriopus japonicus*

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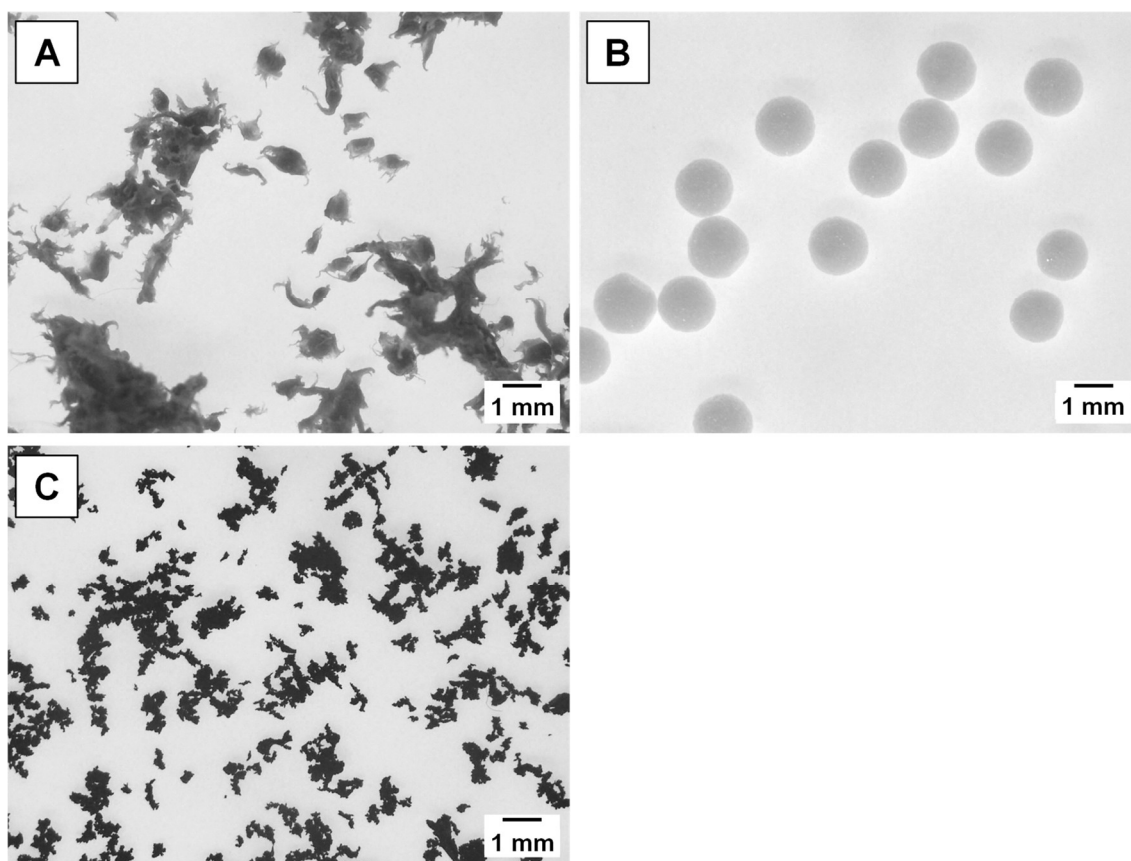


Fig. S1. Photographs of polyethylene pipe fragments (A), expanded polystyrene beads (B), and tire tread particles (C). Scale bar: 1 mm

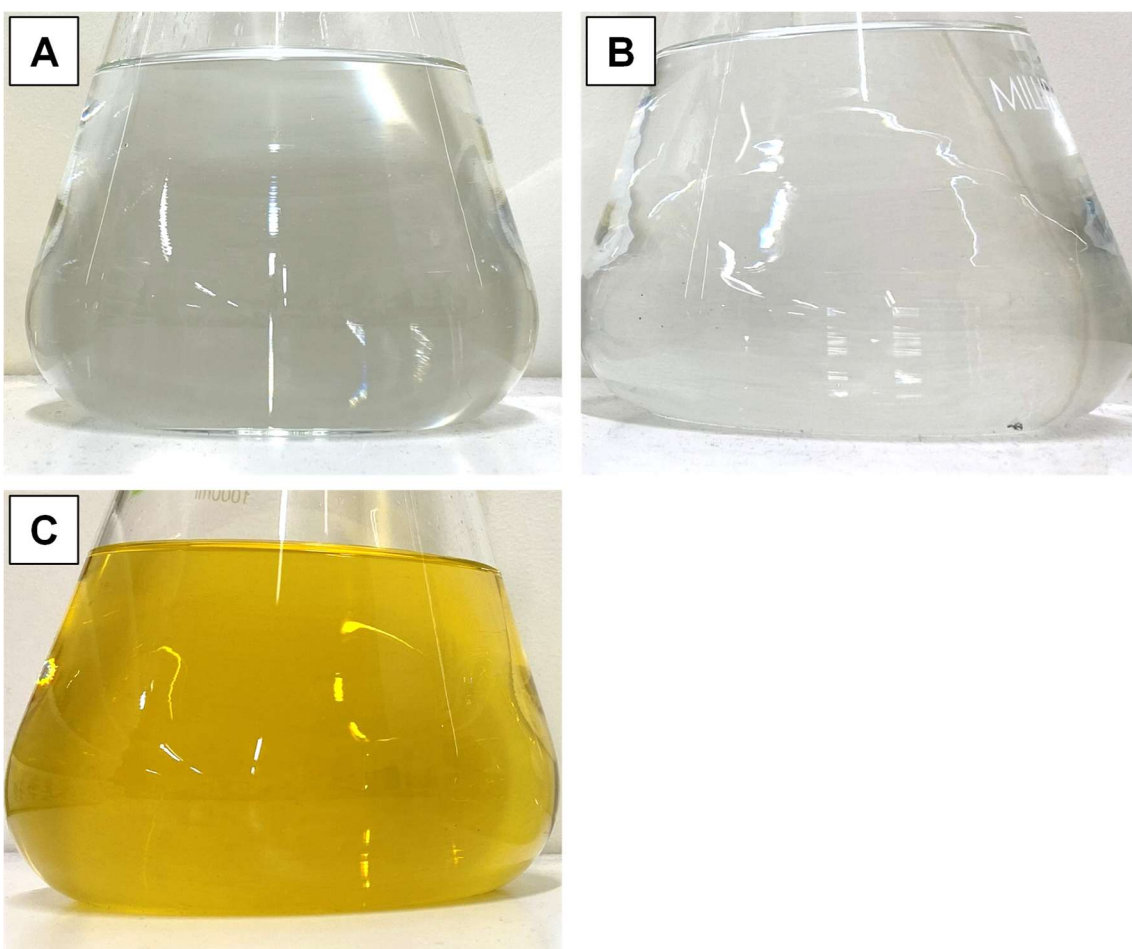


Fig. S2. Color photographs of the leachates from (A) polyethylene pipe fragments, (B) expanded polystyrene beads, and (C) tire tread particles

Table S1. Composition of the ASW-SN+V_{mix} medium. This medium is based on the culture medium for toxicity tests using cyanobacteria described in Yamagishi et al. (2016), with additional vitamins required for the growth of the green algae used in this study. Because the same medium is also used in our laboratory to culture diatoms, silicate was added as well, and we have confirmed that the addition of silicate does not affect the growth of green algae

Substance		Substance		Substance		Substance	
NaCl	25 g	¹⁾ Stock solution (Nutrients)		³⁾ Stock solution (Metal)		⁴⁾ Stock solution (V _{mix})	
MgCl ₂ ·6H ₂ O	2.0 g	NaNO ₃	75 g	Na ₂ EDTA·2H ₂ O	580 mg	Vitamin B ₁₂	2 mg
KCl	0.5 g	NaH ₂ PO ₄ ·2H ₂ O	3.0 g	FeCl ₃ ·6H ₂ O	422 mg	Thiamine HCl	100 mg
MgSO ₄ ·7H ₂ O	3.5 g	Deionized water up to	1,000 ml	ZnSO ₄ ·6H ₂ O	2.93 mg	Biotin	1 mg
CaCl ₂ ·2H ₂ O	0.5 g			CoCl ₂ ·6H ₂ O	1.33 mg	Deionized water up to	100 ml
Nutrients ¹⁾	10 ml			MnCl ₂ ·4H ₂ O	24 mg		
Tris ²⁾	10 ml			Na ₂ SeO ₃	2.3 mg		
Trace metal ³⁾	100 µl	²⁾ Stock solution (Tris)		Na ₂ MoO ₄ ·2H ₂ O	0.839 mg	⁵⁾ Stock solution (Si)	
V _{mix} ⁴⁾	100 µl	Tris	100 g	NiCl ₂ ·6H ₂ O	0.37 mg	Na ₂ SiO ₃ ·9H ₂ O	10 g
Si ⁵⁾	200 µl	Deionized water up to	1,000 ml	Deionized water up to	100 ml	Deionized water up to	100 ml
Deionized water up to	1,000 ml						
pH	8.2						

References

Yamagishi T, Katsumata M, Yamaguchi H, Shimura Y, Kawachi M, Koshikawa H, Horie Y, Tatarazako N (2016) Rapid ecotoxicological bioassay using delayed fluorescence in the marine cyanobacterium *Cyanobium* sp. (NIES-981). *Ecotoxicology* 25:1751-1758. doi: 10.1007/s10646-016-1718-7

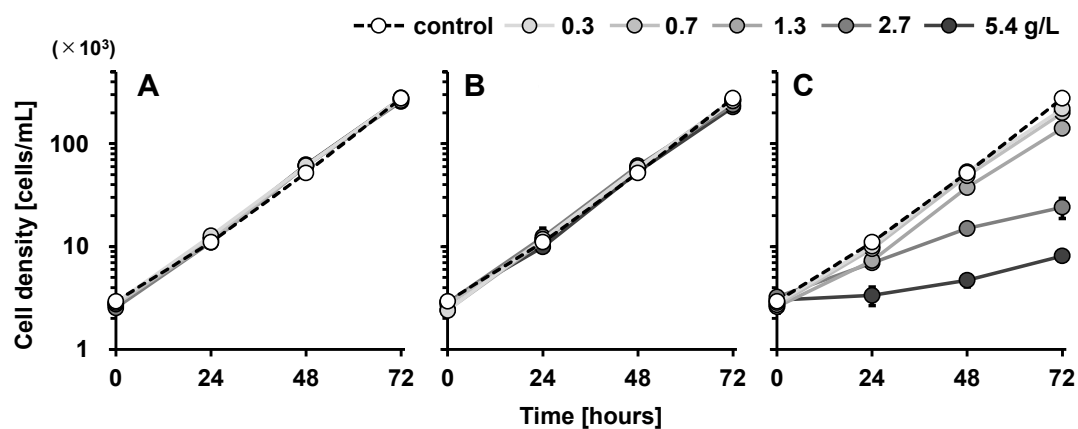


Fig. S3. Growth curves of the microalgae *Dunaliella primolecta* exposed to the three leachates: (A) polyethylene fragments leachate, (B) expanded polystyrene beads leachate, and (C) tire tread particles leachate. Each panel contains six growth curves corresponding to the five concentration levels of the leachate and the artificial seawater control. The legend indicates the particle-equivalent concentrations (g/L), not the dilution ratios of the original leachates

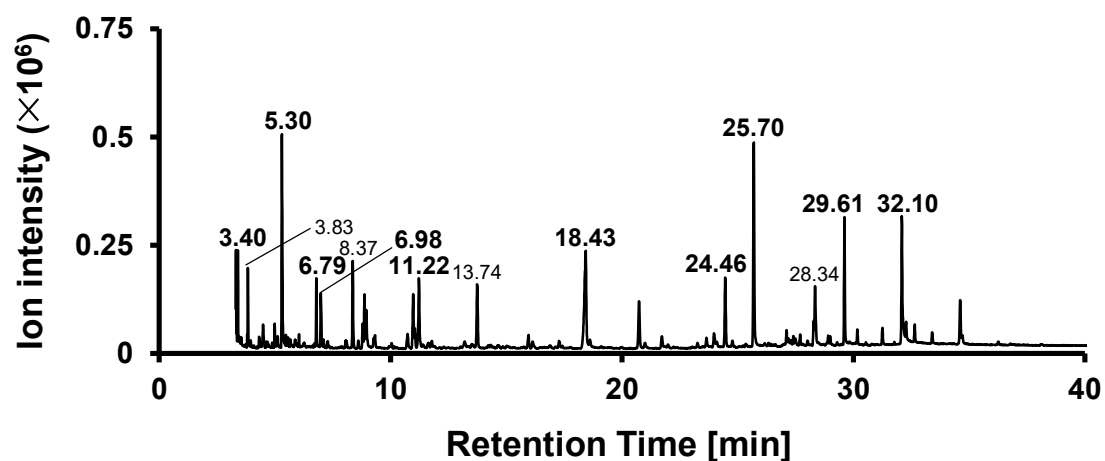


Fig. S4. Total ion chromatograms (TICs) of the background natural seawater analyzed by GC–MS. The ten most intense peaks were selected and labeled with their retention times. Peaks corresponding to the internal standard are indicated in small, thin text, whereas retention times for the top ten non-standard peaks are shown in larger, bold text

Table S2. The ten most intense peaks detected in the GC–MS chromatogram (Fig. S4) of the background natural seawater. Peaks are listed in the order of retention time (RT). Internal standards are indicated by the prefix IS. When an internal standard appeared among the ten highest peaks, it was still included; however, ten non-standard peaks were selected by excluding the internal standards (shown in non-bold text). Compound identifications were based on EI mass spectra from the NIST14 database. Compounds with a match score of 80% or higher, shown in bold, were regarded as reliably identified

RT (min)	Systematic compound name (common name)	CAS number	Concordance rate (%)
3.40	2,2,5,5-Tetramethyltetrahydrofuran	15045-43-9	56
3.83	(IS) 1,4-Dichlorobenzene-d4	3855-82-1	96
5.30	2-(2-Butoxyethoxy)ethan-1-ol (Diethylene glycol monobutyl ether)	112-34-5	80
6.79	2-Methylpropyl 2-methylpropanoate	97-85-8	38
6.98	(3-Hydroxy-2,2,4-trimethylpentyl) 2-methylpropanoate (Isobutyric acid monoester)	77-68-9	80
8.37	(IS) Acenaphthene-d10	15067-26-2	52
11.22	Cadin-4-en-10-ol (α-Cadinol)	481-34	98
13.74	(IS) Phenanthrene-d10	1517-22-2	96
18.43	1-Butyl 2-octyl benzene-1,2-dicarboxylate (Butyl octyl phthalate; BOP) Dibutyl benzene-1,2-dicarboxylate (Dibutyl phthalate; DBP)	84-78-6 84-74-2	90 90
24.46	4-Methoxy-2-[2-(5-nitro-2-pyridinyl)diazenyl]-phenol	1000397-03-8	59
25.70	3-Methylidene-4-prop-1-en-2-ylcyclohexan-1-ol	15358-81-3	60
28.34	(IS) Chrysene-d12	1719-03-5	99
29.61	Bis(2-ethylhexyl) benzene-1,2-dicarboxylate (Bis(2-ethylhexyl) phthalate; DEHP)	117-81-7	91
32.10	(Z)-octadec-9-enamide	301-02-0	64